REMARKS

The Office is authorized to charge the fee for a three month extension of time to Deposit Account No. 02-1818. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

A Change of Correspondence Address accompanies this paper.

A Supplemental Information Disclosure Statement is filed on the same day herewith, under separate cover.

Claims 1-7, 9, 11-16, 45, 48, 50-54, and 56-64 are pending in the application. Claims 1, 2, 7, 16, 45, 53, 54, 57, 59, 61, 63, 64 and 65 are amended, and claims 46 and 47 are cancelled without prejudice or disclaimer. The claims are amended for clarity, to correct antecedent basis, and to address other issues raised by the Examiner. Basis for the amended claims can be found in the specification and in the original and previously pending claims. No new matter is added.

The Examiner indicates that claim that claim 2 is withdrawn. Claim 2, however, is among the rejected claims and claim 2 reads on the elected species of a granzyme B protease. Thus, claim 2 should not be considered withdrawn and is retained herein.

The specification is amended to correct obvious typographical, spelling, punctuation, formatting and grammatical errors. Amendments of the paragraphs beginning at page 22, line 11, correct an obvious error in the Table number referenced within the text. Table 5 clearly shows the specificity of the granzyme B mutants whereas Table 4 lists the actual granzyme B mutations.

I. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45-48, 50-54 AND 56-66 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §112, first paragraph, for alleged lack of written description, because it is alleged that the specification does not describe the subject matter in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the specification was filed. Specifically, the Examiner urges that the disclosure does not describe the huge scope of the claimed components of the broad method steps because it does not provide direction as to which kind of enzyme can be mutagenized, the number of mutations in each protease and the location of the mutations in any of the recited enzymes. This rejection respectfully is traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject matter at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.,* whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir. 1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a parent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443, F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claims need not be described literally (i.e. using the same terms or inhaec verba) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C. 112, first paragraph – description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

The claims

The claims are directed to **methods** of identifying a mutein protease that cleaves a substrate sequence in a target protein involved with a pathology by identifying those protease mutants from among a library of mutants that have increased cleavage activity and/or altered specificity for a substrate sequence in the target substrate. By virtue of the methods the protease muteins are identified. There is no *a priori* requirement to have any knowledge of the location of the mutations or number of mutations in a protease before performing the method. By following the method, such mutant proteases are identified. For example:

Independent claim 1 recites:

> A method of producing and identifying a mammalian protease mutein with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a mammal, wherein:
>
> the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis;
>
> cleavage of a substrate sequence in said target protein serves as a treatment for said pathology; and
>
> the method comprises the steps of:

(a) producing a library of protease muteins of a scaffold, wherein:

each different mutein protease in the library is a member of the library;;

each member of the library has N mutations relative to a wild-type mammalian protease scaffold; and

N is a positive integer;

(b) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence; and

(c) identifying at least one mutein protease having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence in the target protein, relative to the wild-type mammalian protease scaffold.

Dependent claims specify particulars of the method. For example, dependent claim 3-6 specify the N number of mutations in each member of the library of mutant proteases.

Dependent claim 2 specifies that the protease is a cysteine or serine protease and dependent claim 7 specifically recites the starting protease scaffold used in the method. Further independent claims recite as follows:

Independent claim 53 recites:

A method of producing and identifying a mammalian protease mutein with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a mammal, wherein:

cleavage of a sequence in said target protein serves as a treatment for said pathology;

the mammalian protease is selected from among granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin and cruzain; and

the method comprises the steps of:

(a) producing a library of protease muteins each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type mammalian protease scaffold, wherein N is a positive integer;

(b) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence;

(c) identifying at least one protease mutein having increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence relative to the wild-type mammalian protease scaffold;

(d) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step c) as having increased cleavage activity and/or altered specificity for cleaving said substrate sequence;

(e) generating a third mutein protease containing the mutations ~~in a~~ of the first mutein protease and the mutations of the second mutein protease; and

(f) measuring the cleavage activity and/or substrate specificity of the third mutein to determine whether the third mutein produces a protease that has increased cleavage activity toward the substrate sequence and/or altered specificity for cleaving said substrate sequence compared to the first mutein protease or second mutein protease.

Independent claim 59 recites:

A method of identifying a human protease mutein with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a human, wherein:

cleavage of a sequence in said target protein serves as a treatment for said pathology;

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis; and

the method comprises the steps of:

(a) producing a library of protease muteins, each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type human protease scaffold, wherein:

N is a positive integer; and

the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin and cruzain;

(b) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence; and

(c) identifying at least one protease mutein having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence, relative to the wild-type human protease scaffold.

Independent claim 63 recites:

A method of producing and identifying a human protease mutein with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a mammal, wherein:

cleavage of a sequence in said target protein serves as a treatment for said pathology;

the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4); and

the method comprises the steps of:

(a) producing a library of human protease muteins, each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type human protease scaffold wherein:

N is a positive integer; and

the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and plasminogen activator;

(b) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence; and

(c) identifying at least one protease mutein having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence, relative to the wild-type human protease scaffold.

Each of independent claims 53, 59 and 63 recite the specific protease scaffolds used in the method. The dependent claims recite specifics of the methods.

Analysis

The Examiner urges that the claims cover a huge scope of method steps and a huge scope of enzyme and its mutants in a library. The Examiner states that there is no direction regarding which enzymes can be mutagenized, where and with how many mutations, so that the claims do not provide any distinguishing features of the broad claimed method steps and components employed in the method. The Examiner makes reference to differences in substrate specificity between enzymes, the requirement for the catalytic triad in serine proteases (His57, Aps102 and Ser195), that for cysteine proteases the amino acids selected to be modified are less well described and that serine proteases do not have well-defined pockets for substrate recognition. The Examiner concludes that one cannot immediately envisage the genus as claimed based on the disclosure given the early stage of the field.

First, it respectfully is submitted that the instant claims are directed to **methods** of identifying protease muteins, and not to products. Thus, the genus to which the Examiner refers is a genus of methods that include the elements as recited in the instant claims, not a genus of resulting proteases.

The Examiner's comments appear to be premised on the notion that all muteins in the library must be active, and that the method can only be performed if one knows where to generate mutations in a protease to generate an active mutant. This is not correct, and belies the purpose of method as claimed. The method is designed so that it is **not** necessary to know any features required in the resulting protease. If one knew where to introduce mutations into the protease, there would be no need to perform the methods. Since the instant claims are directed to methods of identifying protease muteins, it would be contrary to the purpose of the method for Applicant to describe the location and number of all mutations. Although the specification does describe a rational approach for engineering protease, the

specification also describes random mutagenesis, to generate a diverse library of protease variants. The specification on page 17, lines 16-23, describes the benefits of such a library:

> Also contemplated by the invention are libraries of scaffolds with various mutations that are generated and screened using methods known in the art and those detailed below. Libraries are screened to ascertain the substrate sequence specificity of the members. Libraries of scaffolds are tested for specificity by exposing the members to substrate peptide sequences. The member with the mutations that allow it to cleave the substrate sequence is identified. **The library is constructed with enough variety of mutation in the scaffolds that any substrate peptide sequence is cleaved by a member of the library.** Thus, proteases specific for any target protein can be generated. [emphasis added]

Accordingly, as described in the specification, no knowledge of the location of the mutations is required before performing the method. The claimed methods involve taking a scaffold protease, which can be any protease, preparing a library of muteins, without any requirements where mutations are introduced, and then contacting members of the library with a target protein to identify any members that exhibit increased cleavage activity and/or substrate specificity towards the target compared to the unmodified scaffold. No knowledge of structure/function relationships is required.

It respectfully is submitted that the specification provides sufficient description of proteases that can be used in the method, including methods of generating a library of mutein proteases. As discussed below, the application provides a detailed description of exemplary proteases scaffolds for use in the method and methods of mutating such proteases to generate protease mutein libraries.

1) The specification provides sufficient description of the proteases that can be used in method

The instant claims are directed to methods of identifying a mutein protease that has increased cleavage activity and/or altered substrate specificity for a target protein from among a library of protease muteins, whereby each member of the library has N mutations compared to a wild-type scaffold protease. The specification describes in great detail the scaffold proteases that can be used in the method. The specification describes relevant, identifying characteristics of such proteases, sufficient for use of the proteases in the method as claimed. For example, at page 17, lines 25-32 the specification describes the **process for choosing a scaffold** for use in the method:

> In another embodiment of the invention, scaffold proteases are chosen using the following requirements: 1) The protease is a human or mammalian protease of known sequence; 2) the protease can be

> manipulated through current molecular biology techniques; 3) the protease can be expressed heterologously at relatively high levels in a suitable host; and 4) the protease can be purified to a chemically competent form at levels sufficient for screening.

In addition, the specification describes exemplary proteases for use in the method. For example, Table 2 sets forth many protease scaffolds that can be used in the method. **Notwithstanding this, claims specify the protease** used in the method, and thus provide *prima facie* evidence that Applicant's possessed the claimed subject matter. For example, each of claims 7, 53, 61, 63 and 65 recite specific proteases used in the method. Thus, it is eminently clear that Applicant was in possession of a genus of scaffold proteases, including mammalian and human proteases, that can be used in the methods.

2) The specification provides sufficient description of the number of mutations that can be made in the scaffold proteases and how to generate such mutants

The specification also describes in great detail methods of "producing a library of protease muteins" as described in step a) of the method as claimed. For example, the specification describes that each member of the library is a protease scaffold with a number (N) of mutations made to each member of the library (see e.g., page 2, line 31-32). The specification further details methods for effecting such mutations in the scaffold to **produce a library of protease muteins**. The specification describes that the library can be constructed with enough variation so that any substrate sequence is cleaved by a member in the library (see e.g., page 17, lines 21-22), and that the proteases can include multiple amino acid substitutions (see e.g., page 28, lines 7-8).

In particular, the specification describes a rational mutagenesis approach, whereby the amino acids in the three-dimensional structure that contribute to the substrate specificity are targeted for mutagenesis, *i.e.* those amino acids that are specificity determinants. The specification describes exemplary specificity determinants for serine proteases (see e.g., page 20, lines 3-7; and at page 28, lines 1-3) and also provides structural determinants for various exemplary serine and cysteine proteases (see e.g., Table 3 and Table 6). The specification further describes exemplary granzyme B mutants containing mutations in these specificity determinants (see e.g., Table 4). Under the heading, "Mutagenesis of the Scaffold Protease," the specification describes that mutation of specificity determinants can be effected as follows (see e.g., page 25, lines 3-16):

> In order to change the substrate preference of a given subsite (S1-S4) for a given amino acid, the specificity determinants that line the binding pocket are mutated, either individually or in combination. In one

> embodiment of the invention, a saturation mutagenesis technique is used in which the residue(s) lining the pocket is mutated to each of the 20 possible amino acids... Alternatively single amino acid changes are made using standard, commercially available site-directed mutatgenesis kits such as QuikChange (Stratagene). In another embodiment, any method commonly known in the art ofr site specific amino acid mutation could be used [shortened for presentation herein]

Thus, the specification generically describes methods of mutating a protease randomly or rationally in regions that contribute to substrate specificity. The specification describes the amino acids that are actual specificity determinants in exemplary proteases, describes that such amino acids are targeted for mutation, exemplifies methods of effecting such mutation and exemplifies mutation of specificity determinants in granzyme B. The specification describes that the resulting members in the library can contain a number of mutations such as, for example, 1, 2-5 (*e.g.* 2, 3, 4 or 5), 5-10 (*e.g.* 5, 6, 7, 8, 9 or 10), or 10-20 (*e.g.* 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) (see e.g., page 3, lines 7-9). Accordingly, the specification more then adequately describes methods of producing libraries of protease muteins to perform the method as claimed.

Therefore, it respectfully is submitted that the specification describes in detail the identifying characteristics of protease scaffolds that can be mutated, and provides exemplary proteases. The specification also describes in detail methods for generating libraries of protease mutants. For example, the specification describes in detail that protease muteins can be generated by mutation of the specificity determinants in protease scaffolds. The specification provides the actual specificity determinants in a large number of proteases, including cysteine and serine proteases. The specification further describes methods to effect such mutations, and provides exemplary granzyme B mutants for use in the methods.

Accordingly, it respectfully is submitted that because Applicant adequately describes which kind of enzyme can be mutagenized, and how to effect mutations in order to produce a library of protease muteins, Applicant had possession of the claimed methods, which are for identifying mutein proteases, at the time of filing the application.

Rebuttal to Examiner's Comments

1) *Reliance of In re Ruschig, 379 F.2d 990, 995, 154 USPQ 118, 23 (CCPA 1967) is inapt.*

The Examiner cites In re Ruschig and urges that a laundry list disclosure of every possible moiety does not constitute written description of every species in a genus because it would not "reasonably lead" those skill in the art to any particular species. The issue of the Court in In re

Rushig was whether the compound of claim 13 was supported by the disclosure, whether it was new matter. The disclosure did not disclose the specific compound of claim 13, but instead disclosed a general formula of a compound with specified R groups. It was argued that one of skill in the art could arrive at the claimed compound from the general formula. The Court, however, affirmed the Board of Appeals ruling that the compound was not supported because the compound was "not named or identified by formula and it can find support only as choices made between the several variables involved." Hence, the court concluded that:

> Not having been specifically named or mentioned in any manner, one is left to selection from the myriads of possibilities encompassed by the broad disclosure with no guide indicating or directing that this particular selection should be made rather than any of the many others which could be made.

The claims and disclosure at issue are not analogous to In re Ruschig. In the instant case, Applicant has **not** disclosed a general formula for proteases that can be used in the method, but instead is claiming a method that can employ any protease as a scaffold and has explicitly provided an extensive list of exemplary proteases that can be used in the method. Thus, this is not a "laundry list disclosure of every moiety," but rather a disclosure of the actual proteases contemplated for use in the method. Proteases are described.

In addition, the method, as described, can use virtually any protease, as a scaffold. Mutations are then introduced to produce a library thereof, and the library is then tested to identify member(s) that have increased cleavage of a substrate sequence in a target and/or increased substrate specificity compared to the unmodified protease scaffold. Thus, Ruschig, which was considering an issue of new matter, is inapt.

2) *Applicant respectfully submits that the Examiner's comments regarding caspases are unclear.*

The Examiner states that:

> It seems likely, given the early stage of the field, that more roles exist [for caspase, a cys protease]. Caspases and caspase regulators involved in these processes may be missed in screens that focus strictly on T-5 cell death related phenotypes. Thus, molecules that possess caspase or caspase regulatory activity may not have been identified yet.

The instant claims are directed to methods of identifying protease muteins have increased cleavage activity and/or altered substrate specificity for a substrate sequence contained in a target protein involved in a pathology. The claims are not directed to methods of identifying the role of caspases, nor in identifying caspases or caspase regulators. It is inapposite to the instant claims whether all caspases or caspase regulators have been identified yet. Any protease can be used in

the method, and exemplary proteases are provided. In the method, the protease serves a scaffold into which mutations are introduced. The resulting libraries of proteases are then tested for cleavage activity and/or substrate specificity against a particular target substrate involved in a pathology. The cleavage activity/substrate specificity of the unmodified substrate is not relevant to practice of the methods, since the mutein proteases will have altered cleavage activity/substrate specificity.

In addition, the instant specification describes that caspase-3, which itself is a protease, can be a target protein in the method because it is involved with a pathology, i.e. apoptosis. The specification also provides that other exemplary caspases can be target proteins of the method, e.g., caspse-9, caspase-7, caspase-6 and caspase-2. As target proteins, the caspases themselves are not being modified.

II. REJECTION OF CLAIMS 1, 3-7, 9, 11-16, 45-48, 50-54 and 56-66 UNDER 35 U.S.C. §112, FIRST PARAGRAPH- Scope of enablement

Claims 1, 3-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. 112, first paragraph, because it is alleged that the specification does not reasonably provide enablement for the subject matter as claimed because the specification fails to give adequate direction how to determine the mutations that can be done to a scaffold of any protease to produce a library of muteins, in particular without deleteriously affecting the enzyme-specificity substrate function or its global structure. The Examiner states that the art is inherently unpredictable because it is not possible to predict which predetermined variations of amino acids in different mammalian proteases would result in the desired mutant with a desired pharmacologic activity. The Examiner concludes that one skilled in the art could not be assured that the claim would result in mutations have a pharmacologic activity without undue experimentation. This rejection is respectfully traversed.

As discussed above, the claims are directed to methods of identifying protease muteins that have increased cleavage activity and/or altered substrate specificity towards a target substrate. As discussed above, practice of the methods lead to the identification of protease muteins that have this activity. No knowledge of structure/function is needed, nor is there any requirement that that none of the muteins in the library include mutations that decrease activity or deleteriously affect enzyme-substrate function. As described above, no such knowledge is needed, since the methods identify, from among the members of the library, mutein proteases with increased cleavage activity and/or substrate specificity. If one knew where to introduce mutations in the protease to generate an active mutant that had increased cleavage and altered

substrate specificity compared to a wild-type scaffold, there would be no reason to perform the method. Not all protease variants in the library need be active, functional or even expressed. By virtue of the method, protease muteins are identified that are active based on cleavage activity and substrate specificity for the target substrate. Thus, it respectfully is submitted that the premise upon which the rejection is based is flawed, rendering the rejection moot. Nevertheless, the rejection is discussed in more detail below with reference to the requisite factors demonstrating that it would not require undue experimentation to practice any of the claimed methods.

Relevant law

To satisfy the enablement requirement of 35 U.S.C. §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything within the scope of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of §112, first paragraph "can be fulfilled by the use of illustrative examples or by broad terminology." In re Marzocci *et al.*, 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the subject

matter *as claimed*. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. As discussed in detail below, a consideration of the factors enumerated In re Wands demonstrates that the application, in conjunction with what was known to one of skill in the art as well as other factors, teaches how to make and use the full scope of the claimed subject matter.

Applicant respectfully submits that the instant application teaches protease scaffolds that can be used in the method, teaches how to generate protease mutein libraries thereof, and teaches how to measure activity for a substrate sequence, which are requisite elements of the claims. These and other teachings are provided in the specification and are known to the skilled artisan, as discussed in detail below, and any necessary adjustment can be determined empirically using routine testing. The generation of protease muteins is exemplified with granzyme B. The method is reproducible. Thus, in view of the knowledge and level of skill in the art and the teachings and disclosure in the specification regarding protease scaffolds and methods for producing protease a library of protease muteins thereof, it would not require undue experimentation to practice the method as claimed.

1. The scope of the claims

The claims are directed to a method for producing and identifying a protease mutein from among a library of protease muteins that have increased cleavage activity and/or substrate specificity for a substrate sequence contained in a target substrate that is involved in a pathology. The claims recite specific steps for producing and identifying such muteins, including preparing a library in which each protease mutein member contains N mutations compared to a wild-type scaffold protease, and then identifying from among those members, a protease mutein that has increased cleavage activity and/or substrate specificity compared to the unmodified scaffold protease. No knowledge of structure/function is needed to

produce the library nor to identify a mutein with the requisite increased cleavage activity and/or substrate specificity. Dependent claims 3-6 further specify the number (N) mutations in each member of the library. Independent claim 1 and claim 53 recite that the wild-type scaffold protease is a mammalian protease, and independent claims 59 and 63 recite that the wild-type scaffold protease is a human protease. Each of claims 53, 59 and 63 recite that the protease scaffold is a specifically recited protease scaffold. Claims dependent on claim 1 also further specify the mammalian protease scaffold. For example, claim 7 recites that the mammalian protease scaffold is a particular protease scaffold. As discussed below, each element in the claim is described in the specification.

2. Level of skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art. Therefore, Applicant respectfully submits that using the teachings of the specification, one of skill in the art, could apply such teachings to making domain-exchanged binding molecules.

3. Teachings of the specification

The specification describes methods for producing and identifying protease muetins having increased cleavage activity and/or substrate specificity for a target substrate involved in a pathology as claimed. The specification teaches how to produce a library of protease muteins, each member having N mutations relative to a wild-type protease scaffold; how to measure the cleavage activity and/or substrate specificity of at least two members in the library for the substrate sequence contained in the target substrate; and 3) how identify those having increased cleavage activity and/or altered substrate specificity for the substrate sequence in the target protein. The specification describes in great detail that the method can be performed using any protease muteins of a protease scaffold. The specification describes and provides in detail exemplary protease scaffolds, and describes how to generate mutations in the protease scaffold to produce a library. The application exemplifies practice of the method, including generation of protease muteins, with granzyme B as the scaffold protease.

For example, the instant application teaches that any existing protease can be used as a scaffold, and can be made to contain mutations to change their substrate specificity (see e.g., page 16, lines 6-7). By virtue of the method, those protease that have increased cleavage activity and/or altered substrate specificity compared to a target substrate are identified. The

specification provides teachings of how to choose a scaffold, and teaches that the scaffold is a human or mammalian protease, is one that can be manipulated by molecular biology techniques, can be expressed heterologously at relatively high levels in a suitable host, and can be purified for use in screening (see e.g., page 17, lines 25-32). The specification provides exemplary scaffold proteases that can be used in the method (see e.g, Table 2 and at page 16, line 7-15).

The instant application further teaches methods for mutating such scaffold proteases to generate protease muteins. The specification teaches that various mutations can be made in the library to create a library with enough variety for use in the method (see e.g., page 17, lines 16-23). The specification teaches that the mutations can be made by site-directed mutagenesis (see e.g., page 27, lines 33 to page 28, line 1) or by other known methods of mutagenesis and screening (see e.g., page 28, lines 7-16). The specification also teaches that the protease muteins can be generated by a "structure-based design approach" such that the specificity determinants lining the active site are specifically targeted for mutagenesis. The specification teaches in great detail the specificity determinants of a number of various serine and cysteine proteases (see e.g., page 20, lines 2-6; and Table 3 and Table 6). The specification describes that mutagenesis of those residues can be performed by saturation mutagenesis, such that each active site residue is mutated to every possible amino acid, either alone or in combination with other residues (see e.g., page 28, lines 1-6). The specification exemplifies mutagenesis of active site residues of granzyme B, and provides exemplary mutants (see e.g., page 24, liens 2-7, and Tables 4 and 5).

The specification further teaches methods of testing proteases to measure activity on a substrate sequence. For example, the specification teaches that the protease mutants can be displayed on a phage library for screening against a substrate sequence (see e.g., page 28, line 25 to page 29, line 9), that the protease mutants can be tested using flurogenic peptide substrate sequences that release a fluorogenic moiety upon action of the protease (see e.g., page 29, line 10 to page 31, line 13), or that the proteases can be tested using a protease sequence specificity assay (see e.g., page 31, line 14 to page 32, line 11).

Hence, the specification teaches how to practice all steps of the methods. The specification teaches protease scaffolds including providing an extensive list of exemplary scaffolds, teaches how to mutate such scaffolds to generate protease muteins and libraries thereof, teaches how to measure protease activity to thereby identify proteases that have cleavage activity and/or substrate specificity for the target substrate.

4. Knowledge of those of skill in the art

As of the priority date claimed in this application, a broad body of knowledge was available about proteases, including generating mutants thereof. For example, the sequences of many proteases were known. Also, methods of mutagenesis of proteins, including proteases, using standard recombinant DNA techniques was routine in the art.

For example, such knowledge included availability of structural and sequence information for protease sequences. Such sequence information was available to those of skill in the art in public databases such as Merops database (Rawlings *et al.* (2002) Nucleic Acids Res., 30: 343-6; Barrett *et al.* (2001) J Struct. Biol. 134:95-102. This is evidenced by Table 2 of the specification which provides the Merops code identifier for exemplary proteases (see e.g., merops.sanger.ac.uk/.).

Conventional methods of molecular biology and recombinant DNA techniques also were known to one of skill of the art as of the effective filing date of the instant application. Such techniques are described in numerous books and other references, see, e.g., Sambrook, *et al.* Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Maniatis *et al.* Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984).

Also, at the time of filing the application, molecular biology techniques to effect mutations of a polypeptide sequence were routine. Examples of such well known techniques can be found in Molecular Cloning: A Laboratory Manual 2nd Edition, Sambrook *et al*, Cold Spring Harbor, N.Y. (1989). Examples of conventional molecular biology techniques include, but are not limited to, in vitro ligation, restriction endonuclease digestion, PCR, site-directed mutagenesis, saturation mutagenesis, cellular transformation, hybridization, electrophoresis, DNA sequencing, all of which can be used to practice the subject matter as claimed to generate domain-exchanged binding molecules. The specification cites

Using such techniques, methods of protease engineering, including mutating proteases and generating protease mutant libraries were known to one of skill in the art at the time of filing the instant application (see *e.g.*, Legendre *et al.* (2000) *J Mol. Biol.*, 296:87-102; Demartis *et al.* (1999) J Mol. Biol., 286:617-33); Heinis *et al.* (2001) Protein Eng., 14:1043-52); Bryan PN (2000) *Biochim Biophys Acta*, 1543-203-222; Albert *et al.* (1998) *Adv Exp*

Med Biol., 436:169-77; Chartrain *et al.* (2000) *Curr Opin. Biotech.*, 2:209-214; Lien S *et al.* (1999) Comb Chem High Throughput Screen, 2:73-90; Graham *et al.* (1994) Biochem Mol Biol. Int., 32:831-9; WO98/49286; WO98/11237; US 6110884; US6713281; and US6194183.)

These references to numerous published information and protocols regarding protease sequence, structure, and function including assays used to engineer proteases and generate protease libraries demonstrate the large volume of information regarding tested and reliable procedures available at the time of filing of the instant application. This evidences the advanced state of the art at the relevant time and the availability of such procedures for producing libraries of protease muteins for use in the method as claimed.

5. Presence of Working Examples

The Working Examples exemplify the teachings of the specification with respect to granzyme B, and generation of mutants thereof. Such examples are valuable to demonstrate methods of mutagenesis and methods of screening such mutants for activity.

Example 1 describes generation of mutants of granzyme B using site-directed mutagenesis, and describes expression and purification of the resulting variants. Example 2 describes the synthesis of ACC substrates. Example 3 describes a method for screening of granzyme B mutants against ACC substrate libraries to assess substrate specificity. Example 9 describes the identification of cleavage sites in target proteins that can be used as peptide substrate. Example 7 describes a method for screening of mutant proteases against individual ACC peptide substrates, and Examples 4 and Example 8 describes screening of full-length proteins. Example 11 exemplifies studies measuring activity of a granzyme B mutant for a substrate sequence in caspase-3 using a fluorgenic peptide substrate, a full-length caspase-3, and an in vivo cell assay to measure apoptosis.

6. Nature of the claimed subject matter

The claims are directed to a method of identifying a protease mutein that has increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology. Hence, by virtue of practice of the method, one can identify protease muteins that cleave specific targets proteins involved in a pathology, which can serve as treatment of a pathology. A method of modifying a protease to have specificity for a target protein involved in a pathology in order to identify those that can have therapeutic applications is pioneering in nature. In view of the pioneering nature of the claimed subject matter, Applicant is entitled to claims of relatively broad scope.

7. Predictability

As is known to those of skill in the art (described above), the level of knowledge and skill in proteases and protease engineering was high as of the effective filing date. Using the teachings of the specification and given the level of skill and the knowledge of those of skill in the art, it would not be unpredictable to generate a library of protease muteins, test each member for cleavage activity and/or specificity and identify those that have increased cleavage activity or specificity for a substrate sequence contained in a target protein involved in a pathology. **By virtue of the method, only those proteases that have activity will be identified, and therefore unpredictability regarding whether each protease mutein in the library is active is irrelevant.** As discussed above in great detail, the specification teaches exemplary scaffold proteases, including methods to effect mutations, in particular methods of region-specific mutagenesis of specificity determinants. The specification describes such determinants of specificity for exemplary proteases, and describes that they are shared among families of proteases. Therefore, there is no reason to doubt that one of skill in the art can reproducibly prepare libraries of protease muteins, such as by generating mutant protease that can be measured for cleavage activity and/or substrate specificity for a target substrate involved in a pathology. Thus, unpredictability is not a factor.

Conclusion and Summary

Therefore, based upon consideration of all of the factors, it would not require undue experimentation to prepare protease muteins from a protease scaffold for use in the claimed method. Protease scaffolds and the generation of protease muteins are thoroughly described and taught in the subject specification. The specification describes in extensive detail exemplary protease scaffolds and methods of generating mutants thereof. The level of skill and knowledge of those of skill in the art is high, and the prior art describes all methods needed to practice the methods as claimed in accord with the teachings of the specification. Applicant's discovery of a method for identifying protease muteins with altered specificity and increased cleavage activity for target substrates involved in a pathology has broad and immediate applicability in the field of engineering therapeutic proteases. Applicant is entitled to claims of a scope commensurate with the far-reaching development which has been provided to the public for immediate and valuable use through the guidance of the instant specification.

Rebuttal to Examiner's argument

Reference to Bornscheuer et al. (Curr. Opin. In Chem. Biol.), Harris et al. (PNAS) and Legendre et al. (J. Mol. Biol.) to evidence unpredictability is inapt.

The instant claims are directed to methods, not to products. By virtue of the methods, those proteases that have the desired activity are identified. There is no requirement that all mutant members of the library are active or have the desired activity of increased cleavage activity and/or altered substrate specificity. By virtue of practice of the method those mutants that have the desired activity are identified from among a library of mutants. Thus, it is irrelevant whether minor sequence changes by a single point-mutation may cause significant structural disturbance. If such a mutant is not active, it will not be identified by the method. One does not need to predict which variations of amino acids in a protease would result in the desired activity because it is by practice of the method that such protease muteins are identified.

III. THE REJECTION OF CLAIMS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for various reasons as set forth below. Based on the remarks set forth below, Applicant respectfully requests reconsideration of these rejections.

Relevant law

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject m*att*er permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

Analysis

Claim 1

Claims 1 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that 1) step b) is unclear as to the activity of the protease being measured; 2) there is a lack of nexus between the preamble and step c) of the claimed method because step c) recites identifying the mutein protease relative to the cleavage activity to that of the wild-type protease; and 3) it is not clear whether the reference of N

mutations is with respect to mutations in a single protease or a combination of different proteases.

It respectfully is submitted that as to 1) and 2) above, amendment of the claims herein obviate this rejection. Step b) has been amended to recite that the activity being measured is cleavage activity and/or substrate specificity. Also, the preamble has been amended to recite the elements of step c) in order to provide the nexus between the steps of the method and the preamble. For consistency between and among claims, similar amendments have been made to independent claims 53, 59 and 63.

As to 3) above, it is respectfully submitted that the claim as written is clear because as written the claim recites that each protease in the library has N mutations. For example, step a) of claim 1 recites:

> (a) producing a library of protease muteins, each different mutein protease in the library being a member of the library, **each** member having N mutations relative to a wild-type mammalian protease scaffold wherein N is a positive integer;

Thus, the claim specifies that the library contains more than one protease mutein, and **each** different protease mutein member of the library has N mutations relative to a wild-type mammalian protease.

Claim 7

Claims 7 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that "the mammalian protease scaffold" lacks antecedent basis from the base claim because it is unclear whether it refers to the wild-type or the mutants. It respectfully is submitted that the claim as written is clear because the term "scaffold" provides the necessary antecedent basis with claim 1. Nevertheless, in order to ensure no ambiguity in the antecedent bases, the claim is amended to recite "wild-type mammalian protease scaffold."

Claims 7 and 9

Claims 7 and 9 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that it is unclear from which "among" the protease selection can be made, and further that the term "derived" is unclear. It respectfully is submitted that this rejection is moot as to the term "derived," which has been deleted from claim 7. As to the first reject for the term "among," Applicant respectfully requests clarification. There is only one "among" recited in each of claim 7 and 9. Thus, the claims are clear that the method can be performed with a scaffold that is any one of the proteases recited in claim 7.

In addition, the substrate sequence can be one in a target protein that is involved in any of the recited pathologies of claim 9.

Claim 12

Claim 12 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite in the recitation of caspase as the target because the base claim does not recite an enzyme as the target. It respectfully is submitted that claim 1 is directed to a method of identifying a mutein protease that has increased cleavage activity and/or altered substrate specificity for a target protein. Caspase-3 is a protein, since proteases are proteins. Hence, it respectfully is submitted that claim 12 is clear as written.

Claim 16

Claim 16 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that steps d) and e) are unclear as to how two or more members of the protease library identified with increased cleavage activity are provided. In addition, it is alleged that it is unclear whether two simultaneous mutations are being done on the same or different libraries. The Examiner concludes that it appears that the base claim is broadened.

It respectfully is submitted that this rejection is obviated based on amendment of the claim herein. The phrase "provided" has been deleted from the claim. In addition, the claim is amended to render it clear that the mutations are to a third mutein protease and not on a library of proteases.

Also, the Examiner is reminded that a dependent claim does not have to further limit the claim from which it depends, but rather must include all limitations in the claim from which it depends. As stated in MPEP (608.01(n), the test for a proper dependent claim is:

> **Whether the dependent claim includes every limitation of the parent claim. The test is <u>not</u> whether the claims differ in scope. A proper dependent claim shall not conceivably be infringed by anything which would not also infringe the basic claim.**

A dependent claim does not lack compliance with 35 U.S.C. 112, fourth paragraph, simply because there is a question as to (1) the significance of the further limitation added by the dependent claim, or (2) whether the further limitation in fact changes the scope of the dependent claim from that of the claim from which it depends. The test for a proper dependent claim under the fourth paragraph of 35 U.S.C. 112 is whether the dependent claim includes every limitation of the claim from which it depends. The test is not one of whether the claims differ in scope. [see MPEP 608.01(n)].

Thus, claim 16 is properly dependent because it includes every limitation of the base claim. In addition to steps a)-c) set forth in claim 1, claim 16 recites that the method further comprises the steps of:

> (d) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step c) as having increased cleavage activity and/or altered specificity;
>
> (e) generating a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein protease; and
>
> (f) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine it has an increased cleavage activity and/or altered specificity for the substrate sequence compared to the first mutein protease or second mutein protease.

The method of claim 16 necessarily includes the method steps of claim 1. Therefore, claim 16 includes all limitations of the base claim and is properly dependent.

Claims 45 and 57

Claims 45 and 57 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged the claims are unclear as to whether in repeating the steps a new library is being created, and that the term "selectivity" lacks antecedent basis in the base claim. This rejection is obviated by amendment of the claim herein. The claim is amended to specify that the steps are repeated by producing a library from a protease identified in step c) to identify a "further mutein protease." Accordingly, the amendment renders it clear a new library is generated, and the steps repeated, to identify a further mutein protease. With respect to the term "selectivity," this issue is moot with by amendment of the claims herein to delete the term.

Claim 46

Claim 46 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that it is unclear to which "earlier iteration of the method" is being referred to. This rejection is moot by cancellation of the claim herein.

Claim 47

Claim 47 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that the term "corresponding" wild-type protease is unclear. This rejection is rendered moot by cancellation of claim 47 herein.

Claim 53

Claim 53 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for reasons stated above with respect to claims 1, 7, 9 and 12. For example, the Examiner alleges that step b) is unclear as to the activity of the protease being measured; 2) there is a lack of

nexus between the preamble and step c) of the claimed method because step c) recites identifying the mutein protease relative to the cleavage activity to that of the wild-type protease; and 3) it is not clear whether the reference of N mutations is with respect to mutations in a single protease or a combination of different proteases. For the reasons discussed above with respect to claim 1, it respectfully submits that amendments to claim 53 also obviate this rejection.

In addition, the Examiner alleges that it is unclear as from which "among" the protease selection can be made and also that the term derived is unclear. With respect to the latter, the term "derived" is not set forth in this claim, and hence this rejection is not apt. With respect to "among," as discussed above with respect to claims 7 and 9, Applicant requests clarification of this rejection. There is only one recitation of "among" in the claim in referring to the alternative proteases. Thus, Applicant submits that it is clear that the method can be performed with any of the recited proteases.

Claim 63

Claim 63 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for various reasons. First, the Examiner alleges that the claim omits essential structural cooperative relationships of elements and essential steps, in particular the steps between steps a) and b). The Examiner states that it is not clear as to how step b) can occur given no step for providing a substrate or a step by which an enzyme cleaves a substrate sequence. Second, the Examiner alleges that recitation of both human scaffold protease and mammalian scaffold protease is inconsistent. Third, the Examiner alleges that it is not clear as to the difference between identifying a protease mutein having an "increased" cleavage activity from an "altered" activity, especially in the absence of positive differentiating steps between the two.

As to the second rejection, this issue is moot by amendment of the claim to recite "human protease scaffold" in all instances.

As to the first rejection, it respectfully is submitted that the claim as pending is clear and that the steps can be performed in the order shown. For clarity, the claim is amended by moving the list of recited target proteins in step b) immediately below the limitation referring to the target protein as being one involved in a pathology. As amended, step a) and b) of the method are as follows:

(a) producing a library of human protease muteins, each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type human protease scaffold wherein:

N is a positive integer; and

the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and plasminogen activator;

(b) measuring a cleavage activity and/or substrate specificity of at least two members of the library towards the substrate sequence

Thus, in performing the method as claimed, a library of human protease muteins is generated, each member in the library containing N mutations relative to a wild-type human protease scaffold set forth in the recited list. Then, at least two or more members of the library are assessed for cleavage activity and/or substrate specificity towards a substrate sequence contained in a target protein involved in a pathology. The target proteins are recited in the claim. Accordingly, all steps of the method are recited.

As to the third rejection, it respectfully is submitted that "cleavage activity" and "substrate specificity" are two separate measures of cleavage of the substrate sequence that can be performed in the method. Thus, step c) of the method identifies those mutants that have an "increased" cleavage activity and/or and "altered" substrate specificity.

Claim 64

Claim 64 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that the claim lacks antecedent basis for "human protease scaffold" recited in claim 63. In addition, the Examiner comments that the claim appears to be duplicative. This issue is moot by amendment of claim 64 herein to recite "human protease scaffold." In addition, it respectfully is submitted that the claim is not duplicative. From among "increased cleavage activity" and "altered substrate specificity" recited in claim 63, claim 64 recites that the protease is identified based on "altered substrate specificity. Thus, claim 64 further limits claim 63.

IV. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45-48, 50-54 and 56-66 UNDER 35 U.S.C. §103

Claims 1-7, 9, 11-16, 45-48, 50-54 and 56-66

Claims 1-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §103(a) as being unpatentable over Harris *et al.* I (The Journal of Biological Chemistry) or Harris *et al.* II (Current Opinion in Chemcial Biology), each alone, and over Harris *et al.* I or II in view of Bianchi *et al.* (Bioplymers) because Harris *et al.* I and II are alleged to teach a method of

identifying optimal substrate specificity for proteases such as granzyme B that allow for the identification of in vivo substrates in the process, and Bianchi *et al.* teaches advantages in the use of combinatorial library of enzymes. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to substitute the substrate library of Harris with the enzyme library of Bianchi and that the advantages taught by Bianchi would motivate one to do so. Further, the Examiner concludes that the different variants of granzyme B taught in Harris *et al.* could lead one to a combinatorial library of said enzyme. This rejection respectfully is traversed.

The Examiner did not provide complete citations for the cited references, which Applicant respectfully requests. In the interests of advancing prosecution, however, it is assumed herein that Harris *et al.* I is *J. Biol. Chem.*, 273: 27364-27373 (1998)), Harris *et al.* II is Current Opinion in Chemical Biology, 2:127-132 (1998)), and Bianchi *et al.* is Biopolymers (Peptide Science), 66 :101-114 (2002). If this is incorrect, issuance of a new, non-final Action setting forth the citations, respectfully is requested.

Relevant Law

To establish prima facie obviousness under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was not altered by the recent Supreme Court holding in KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). See also KSR, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the

circumstances surrounding the origin of the subject matter sought to be patented." 383 U.S. at 18, 148 USPQ at 467. Furthermore, the Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Id. at 1740-41, 82 USPQ2d at 1396 (citing In re Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. KSR, 127 S. Ct. at 1731. The court stated in dicta that, where there is a "market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try **might** show that it was obvious under § 103."

In a post-KSR decision, PharmaStem Therapeutics. Inc. v. ViaCell. Inc., 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled See In re Papesch, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and In re Deuel (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

As always, unexpected properties must always be considered in the determination of obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesch*, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963)

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. In re Laskowski, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The Rejected Claims

The claims are directed to **methods** for producing and identifying a protease mutein with *increased cleavage activity* and/or *altered substrate specificity* for **a target protein involved with a pathology.**. For example, independent claim 1, 53, 59 and 63 recite specific steps . The method includes the steps of producing a producing a library of protease muteins of the scaffold, where different member has N mutations relative to the wild-type protease scaffold. Cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence in the target is measured. At least one mutein protease having an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence, relative to the wild-type mammalian protease scaffold is identified. Dependent claims recite particulars of the methods.

Independent claims 53 and dependent claims recite additional steps of the method to identify further mutein proteases. Independent claims 1, 59 and 63 also specify target proteins. Independent claims 53, 59, and 63 and dependent claims 2, 7 specify scaffold

proteases. *For example, each of independent claims 59 and 63, and claims dependent thereon, are specific to methods using* ***human*** *scaffold proteases from among a list of recited scaffold proteases, and identifying human protease muteins.* Hence, all claims are directed to methods of discovery of protease muteins, human or mammalian, that have increased cleavage activity or substrate specificity for a substrate sequence in **a target protein in a pathology** compared to a starting scaffold protease not containing the N mutations, such that cleavage of the target protein results in treatment of the pathology. All claimed methods employ **a library of proteases muteins** based on a particular scaffold that include mutations. Identified proteases are those that have increased cleavage activity and/or substrate specificity for the target protein.

Differences Between the Claims and Teachings of the Cited References

Harris *et al.* I (*J. Biol. Chem.*, 273: 27364-27373 (1998

Harris *et al.* I is directed to the screening combinatorial **substrate** libraries to identify *in vivo* substrates of **rat** granzyme B in order elucidate its substrate specificity. Using the combinatorial substrate libraries, Harris *et al.* I teaches identification of the optimal P4-P2 substrate specificity profile of granzyme B. Harris *et al.* I teaches the optimal P4 to P2' granzyme B cleavage site to be (Ile>Val)(Glu>Gln= Met)Xaa-Asp/ Xaa-Gly, for example, the substrate sequence IEPD/. Harris *et al.* also teaches identification of *in vivo* targets of granzyme B based on the elucidation of the substrate specificity of granzyme B. For example, Harris *et al.* teaches that based on the substrate specificity of granzyme B, certain caspases (caspases 3 and 7) , based on their sequences, are more likely substrates then other caspases. Harris *et al.*, also teaches that based on the sequence specificity of granzyme B, nuclear lamin A and nuclear poly(ADP)ribose polymerase (PARP) are potential *in vivo* substrates for granzyme B. Harris *et al.* I also teaches that amino acid position Arginine 192 is a structural determinant of specificity of granzyme B, since granzyme B mutations R192E and R192A exhibit reduced hydrolysis of the optimal tetrapeptide substrate Ac-IEPD-AMC and non-optimal tetrapeptide substrate Ac-IKPD-AMC compared to the wild-type enzyme.

Harris *et al.* is of no relevance to the instant claims, since it fails to teach or suggest **any** element of the instantly claimed methods. For example, Harris *et al.* does not teach modification of substrate specificity, nor a method that includes providing a library of modified proteases based on a particular scaffold, as required by the instant claims.

Harris *et al.* I does not teach or suggest a method of identifying mutein proteases, including mutein granzyme B proteases, that have increased cleavage activity and/or altered specificity for a

target protein involved in a pathology. There is no teaching or suggestion in Harris *et al.* I of a method that includes a step of preparing a library of muteins of a particular protease scaffold, such as granzyme B; then measuring the cleavage activity or substrate specificity of **granzyme B muteins** in the library for **a substrate sequence in a target protein involved in a pathology;** and then identifying a library member that has increased cleavage activity and/or substrate specificity for the target compared to the wild-type protease.

Thus, Harris *et al.* I is deficient in failing to teach or suggest any elements of the instantly claimed methods. The instant claims are directed to a method of producing and identifying protease muteins by preparing a library of protease muteins, and identifying from the library any protease that have increased cleavage activity and/or altered substrate specificity for a selected target protein involved in a pathology. Harris *et al.* I fails to teach or suggest preparing a library of mutein proteases; assessing cleavage activity and/or substrate specificity for a particular target protein; and identifying any in the library that has increased cleavage activity and/or substrate specificity for the substrate sequence in the target protein compared to the scaffold protease not containing the N mutations for the target. Thus, Harris *et al.* is of no relevance.

Harris *et al.* II (Current Opinion in Chemical Biology, 2:127-132 (1998))

Harris *et al.* II is a review article directed to methods for determinants of protease specificity and for modifying specificity as a means to "understand the factors involved in protease specificity." Harris *et al.* II teaches that several methods that have been employed to make changes in enzyme specificity in order to understand protein design principles. The first method, designated rational redesign specificity, requires a detailed understanding of the catalytic mechanism and sequence determinants for a particular protease as basis for predictably altering specificity. Harris *et al.* II also teaches complete random mutagenesis and states that this requires large libraries of mutants to identify a desired function. Another method, employs comparative analysis using homologous proteins that differ in substrate specificity. In another method, describes the use of alanine scanning to identify residues involved in activity, followed by replacement of identified residues with all 19 amino acids to produce modified proteins with altered activity. In a final method, Harris *et al.* teaches using directed evolution, such as DNA shuffling methods, to generate enzymes with modified activities.

None of the methods include steps in which protease that has increased cleavage activity and/or substrate specificity for a particular target by preparing a library of muteins of a

particular protease whose cleavage activity and/or substrate specificity is to be modified and identifying from among the muteins any that have increased cleavage activity and/or substrate specificity for the target. None of the methods of Harris *et al.* II include any or all of these required elements. Harris *et al.* II is a review article that describes methods that have been used to identify structural determinants. None of the methods described by Harris *et al.* are for generating a protease that has specificity for a particular target involved in a pathology. None of the methods include the steps required by the instant claims. The methods described by Harris *et al.* provides a general review of the state of the art at the time of its publication.

Harris *et al.* II does not teach or suggest a method of producing and identifying a protease with **increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology**. There is no teaching or suggestion in Harris *et al.* that a protease having increased cleavage activity and/or substrate specificity for a target protein in a pathology could be used as a treatment. Hence, there is no teaching or suggestion in Harris *et al.* of modifying a protease to have increased cleavage activity and/or specificity for a substrate sequence in a target protein involved in a pathology nor any methods of doing so. Hence, Harris *et al.* II alone fails to teach or suggest any elements of the the instantly claimed methods, and thus, the combination of Harris *et al.* I and Harris *et al.* II fail to teach or suggest any elements of the instantly claimed methods.

Bianchi *et al.* (Biopolymers (Peptide Science), 66 :101-114 (2002))

Bianchi *et al.* fails to cure the deficiencies in the teachings of Harris *et al.* I and Harris *et al.* II. Bianchi *et al.* is directed to the a review of studies on human hepatitis C virus (HCV), and identification and mechanism of action of inhibitors thereof. Bianchi *et al.* teaches strategies to identify inhibitors of human hepatitis C virus (HCV), specifically the nonstructural NS3 proteins, and discusses that knowledge of the broad substrate specificity of enzymes should be taken into account to develop inhibitors against viral proteases such as NS3. Bianchi *et al.* teaches that NS3 is inhibited by its own cleavage products, and from the elucidation of known inhibitors of NS3 describes combinatorial libraries of inhibitors to identify inhibitors of enzymes that exhibit conformational flexibility due to a viruses ability to rapidly change in respond to its environment.

Bianchi *et al.* is of no relevance to the instant claims; Bianchi *et al.* fails to teach or suggest any elements of the instantly claimed methods. For example, there is no teaching or suggestion in Bianchi *et al.* directed to using a particular protease as a scaffold for producing a mutein protease with increased cleavage activity and/or substrate specificity for any target, including one involved in a pathology such that cleavage would serve as a treatment for the pathology. Bianchi *et al.*

fails to teach or suggest the generation of protease muteins of the particular protease nor libraries such muteins. The instant claims are directed to methods of producing and identifying proteases muteins against a target substrate involved in a pathology. Hence, the instant claim require a library of protease muteins. Bianchi *et al.* employs a combinatorial libraries of **inhibitors**. Thus, Bianchi *et al.* cannot teach or suggest generating a protease with altered specificity by producing a library of mutein proteases and identifying from among the library members any that have increased cleavage activity and/or substrate specificity compared to the unmodified protease. There is no teaching or suggestion of any method of evolving a protease scaffold to produce and identify muteins, in particular to identify muteins having increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology. Bianchi *et al.* fails to teach or suggest any steps of the instantly claimed methods. Hence, Bianchi *et al.* does not cure the deficiencies in Harris *et al.* I or II. Therefore, the Examiner has failed to set forth a case of *prima facie* obviousness.

Analysis

The combination of teachings of the cited references does not result in the instantly claimed methods.

The teachings of the references singly or in combination fails to teach or suggest all elements as claimed. Each of independent claims 1, 53, 59 and 63 is directed to a method of producing and identifying a protease mutein that has increased cleavage activity and/or altered substrate specificity for a substrate sequence in a **target protein involved in a pathology** compared to the unmodified protease. The specific steps of the methods include: producing a library of protease muteins of the scaffold proteases, measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence that is one that is present in **the** target protein involved in a pathology, and identifying a protease mutein(s) that has increased cleavage activity and/or altered substrate specificity for that target compared to a wild-type scaffold protease not containing the modifications. There is no teaching or suggestion in any fo the cited art, singly or in any combination thereof, and any steps or elements of the claimed methods. None of the cited references teaches or suggests any method for producing and identifying protease muteins of a scaffold protease that have increased cleavage activity and/or altered substrate specificity for partiuclar target protein involved in a pathology compared to the unmodified protease. None of the cited references, singly or in any combination, teaches or suggests a method of identifying a protease mutein that cleaves a specific protein involved in a pathology, whereby cleavage of the target protein serves as a treatment of the pathology.

As discussed above, Harris *et al.* I teaches elucidation of the substrate specificity of wild-type **rat** granzyme B, and the identification of *in vivo* substrates based on knowledge of the substrate specificity. Harris *et al.* I teaches that position 192 is an important amino acid determinant of substrate specificity, and that mutation of the site **reduces** the ability of the enzyme to hydrolyze both an optimal and non-optimal substrate. There is no teaching or suggestion for using granzyme B as a scaffold produce a protease with increased its cleavage activity and/or altered its substrate specificity for any target, nor any methods of doing so. There is no teaching or suggestion of identification granzyme B muteins that cleave target proteins involved in a pathology, nor of methods of doing so.

Harris *et al.* II does it cure the deficiences in Harris *et al.* I. Harris *et al.* II is a general review of the art of engineering proteases. Harris *et al.* II teaches various known methods of engineering proteases. None of the teachings in Harris *et al.* II teach or suggest any elements of the claimed methods nor any methods of identifying protease muteins that cleave a target protein in a pathology, by identifying protease muteins that have increased cleavage activity and/or altered substrate specificity for a substrate sequence in the target protein.

Bianchi *et al.* does not cure the deficiencies in the teachings of Harris *et al.* I or Harris *et al.* II. Bianchi *et al.* is of no relevance to the instant claims. It is directed to **combinatorial libraries of inhibitors** to identify inhibitors of viral proteases. The instantly claimed methods employ libraries of protease muteins based on a particular scaffold protease to identify muteins that have increased cleavage activity and/or target specificity for a target protein involved in a pathology. Bianchi *et al.* does not teach or suggest any modified proteases, any methods of modifying proteases, norr any method to identify a protease mutein that cleaves a target protein involved with a disease. There is no teaching or suggestion in Bianchi *et al.*, when combined with any of the other cited references, that would result in the method as claimed for producing and identifyingo identify protease muteins that have increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology compared to the unmodified scaffold.

Thus, the combination of teachings of the cited references fails to teach or suggest the any elements of the independent claims, including: 1) producing a library of protease muteins of a scaffold protease that have N mutations compared to a wild-type protease scaffold; 2) measuring the cleavage activity and/or substrate specificity for a substrate sequence contained in a target protein involved in a pathology; and 3) identifying a protease mutein having increased cleavage activity and/or substrate specificity for the substrate sequence in the target protein in order to produce and identify a protease that has increased increased cleavage activity and/or altered

substrate specificity for the target compared to the unmodified scaffold. The references, singly or in combination, also fail to teach other elements as claimed. For example, none of the references, singly or in any combination, teaches or suggests the recited target proteins and/or scaffolds; none, singly or in any combination, teaches or suggests producing and identifying a modified human protease mutein based on the human protease scaffold as recited in claims 59 and 63; none of the references, singly or in any combination, teaches or suggests further steps involving combining mutations from identified proteases or iteratively repeating the methods. Since a *prima facie* case of obviousness requires that the references, singly or in any combination, teach or suggest all elements as claimed, the Examiner has failed to set forth a case of *prima facie* obviousness.

Hence, none of the references, singly or in any combination, teaches or suggests a method of identifying protease muteins that cleave a target protein involved in a disease by producing a library of protease muteins, measuring the cleavage activity or substrate specificity towards a substrate sequence in a target protein involved in a disease, and identifying those protease mueteins that have increased cleavage activity and/or altered substrate specificity for the substrate sequence. None of the references, singly or in any combination, teach particulars of the method, such as the specific target proteins and scaffold proteases used in the method. The combination of the teachings of Harris *et al.* I, Harris *et al.* II and Bianchi *et al.* do not result in the instant claims. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 16, 45-46 and 53

The Examiner states that Claims 16, 45-46 and 53 are rejected "over the disclosure of Harris *et al* because of the known iterative process of phage display method." It respectfully is submitted that it is not clear to which Harris *et al.* reference the Examiner refers, nor is the nature of the rejection clear. In the interests of advancing prosecution, the remarks below are directed to Harris *et al.* I and Harris *et al.* II. Further, it is assumed that this is not a rejection that is separate from the rejection addressed above, but rather is intended to point out elements of dependent claims. Nevertheless, this rejection respectfully is traversed for the reasons discussed above with respect to the independent claims.

The Claims

Each of claims 16 and 45-46 are dependent on claim 1. Claim 1 is set forth above. Each of dependent claims 16 and 45 recite further steps of the method. Claim 16 recites that the method further comprises the steps of identifying the mutations contained in a first and second identified protease as having increased cleavage activity and/or altered substrate specificity, and combining the mutations to generate a third mutein protease. Claim 45 is directed to a further iterative

method, whereby the steps of the method of claim 1 are repeated by generating a further protease library from an identified protease mutein, and repeating the steps to identify a further mutein with increased substrate specificity or increased cleavage activity compare to a wild-type mutein protease. Hence, each of claims 16 and 45 require the steps of claim 1.

Independent claim 53 is directed to a method of identifying a mutein protease with increased cleavage activity and/or altered substrate specificity, and includes the same steps as claim 1. Claim 53, recites specific scaffold proteases used in the method. In addition, claim 53 recites further steps of identifying the mutations contained in a first and second identified protease as having increased cleavage activity and/or altered substrate specificity, and combining the mutations to generate a third mutein protease.

The references

Each of Harris *et al.* I and Harris *et al.* II are discussed above. It respectfully is submitted that neither Harris *et al.* I nor Harris *et al.* II teaches or suggests an iterative phage display method that displays mutein proteases. Harris *et al.* I teaches a **substrate** phage display, but does not teach or suggest any method for identifying protease muteins, nor any iterative method for identifying protease muteins, in particular protease muteins having an increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology. Harris *et al.* II also does not teach or suggest a method of identifying further protease muteins by first identifying those that have increased cleavage activity and/or altered substrate specificity for a substrate sequence in a target protein involved in a pathology, and then either combining the mutations of two of the identified protease(s) to generate a third protease or repeating the method by producing a new library from an identified protease, whereby the further protease muteins have increased cleavage activity and/or altered substrate specificity towards the substrate sequence. Thus, Harris *et al.* I and II, alone or in combination, fail(s) to teach all elements as claimed. Bianchi *et al.*, as discussed above fails to cure these deficiencies.

Analysis

The Examiner has failed to set forth a prima facie case of obviousness because none of Harris *et al.* I, Harris *et al.* II and/or Bianchi *et al.*, singly or in any combination thereof teaches all elements as claimed. Each of claims 16 and 45 is dependent on claim 1. Claim 53 also includes the elements of claim and further steps. Thus, as discussed above, insofar as Harris *et al.* I and Harris *et al.* II (and Bianchi *et al.*,) singly or in any combination fails to teach or suggest any elements of the method of claim 1, these references fail to teach or suggest any elements of claims 16, 45 and/or 53. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 1, 2-7, 9, 11-16, 45-48, 50-54 and 56-66

Claims 1, 2-7, 9, 11-16, 45-48, 50-54 and 56-66 are rejected under 35 U.S.C. §103(a) as being unpatentable over Kolterman *et al.(* (US 2004/0072276) in view of Waugh *et al.* (Nature Structure Biology) because it is alleged that Kolterman *et al.* teaches a method for generating sequence-specific proteases, and Waugh *et al.* discloses that Granzymes are a vital component of cytotoxic lymphocytes ability to induce apoptosis by cleavage of downstream caspases. The Examiner concludes that it would have been obvious to one of ordinary skill to use granzyme as the enzyme as taught by Waugh in the method of Kolterman, and that one would be motivated to do so because of the advantage taught by Waugh that granzyme leads to rapid cell death of a tumor cell. This rejection is respectfully is traversed.

It respectfully is submitted that the Examiner did not provide complete citations for the cited reference of Waugh *et al*, which Applicant respectfully requests. In the interests of advancing prosecution, however, it is assumed herein that the Waugh *et al.* reference relied upon is Waugh *et al.*, Nature Structure Biology 7: 762-765 (2000).

The Rejected Claims

The claims are discussed above. Each of independent claims 1 and 59 recite that the target protein is a protein that is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis. Independent claim 63 recites that that the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4);. Dependent claims recite particulars of the method.

Independent claim 53, which includes the same method steps as independent claim 1, does not recite particular target proteins, but requires that the target protein is involved in a pathology and recites further steps of the method to identify a third mutein protease that is generated by

combining the mutations of mutein proteases identified in the method. Claim 53 also recites specific scaffold proteases used in the method to generate a library of protease muteins. Dependent claims recite particulars of the method.

Differences Between the Claims and Teachings of the Cited References

Kolterman *et al.*((US 2004/0072276)

Kolterman *et al.* is directed to a method for generating a sequence-specific protease that recognizes and cleaves a user-definable amino acid sequence with high specificity, such that only cleavage of specific amino acid sequences occurs. The method involves contacting a population of protease variants specific for a first substrate with a second substrate that contains a specific amino acid sequence resembling the target peptide substrate and selecting one or more protease variants that has specificity preferably for the second substrate. Kolterman *et al.* does **not** teach or suggest *any specifics of the second substrate, nor of the target peptide substrate*, except to provide as a preferred target substrate a substrate of tissue-type plasminogen activator (CPGR/VVGG). In some examples of the method, Kolterman *et al.* teaches that that the substrate has an intermediate sequence compared to the first substrate and the second substrate. In other examples of the method, the second substrate containing **the target peptide sequence is incubated with the protease in the presence of an inhibitor.** Kolterman *et al.* teaches that steps of its method can be repeated until one or more protease variants with specificity for the intermediate substrate and/or the target substrate are identified. Kolterman *et al.* teaches exemplary proteases that can be used in the method, in particular BAR1 protease.

Kolterman *et al.* does not suggest producing a library of protease muteins of a protease scaffold, where each member of the library has N mutations relative to the wild-type protease scaffold. The library of Kolterman *et al.* contains protease variants **specific for a first substrate**. Thus, the library used in the method of Kolterman *et al.* is different from the library used in the instantly claimed methods. Furthermore, the library is contacted with a second substrate that contains a specific amino acid sequence resembling the target peptide substrate or with a substrate sequence of a target, but in the presence of an inhibitor. In contrast, in the instantly claimed methods, the library is contacted with a target, and the target is one involved in a pathology. The method of Kolterman *et al.* then includes a step of selecting one or more protease variants that has specificity preferably for the second substrate. In contrast, the instantly claimed methods include a step of identifying at least one mutein protease that has increased cleavage activity and/or altered substrate specificity for

cleaving a substrate sequence in the target protein compared to the unmodified protease scaffold. Thus, each of the recited steps in the method of Kolterman *et al.* differs substantially from each of the steps of the instantly claimed methods.

Furthermore, Kolterman *et al.* does not teach or suggest the additional elements of the instant claims. Kolterman *et al.* does not teach or suggest that the target substrate used in the method is a target substrate involved with a pathology, and particularly a target among the recited targets in independent claim 1, 59, 63 and/or 66. There is no teaching or suggestion of any target substrates used in the method, except a substrate of tissue plasminogen activator.

Kolterman et al. does not teach or suggest a method in which the target substrate is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis, nor any specific proteins recited in independent claims 1, 59 and/or 63. Kolterman et al. does not teach a method containing a step of identifying protease muteins that have increased cleavage activity and/or altered substrate specificity for the substrate sequence in the target substrate. Kolterman et al. also does not teach a step of the method of combining mutations from identified mutein proteases to generate a third protease, and then measuring the cleavage activity and/or substrate specificity thereof to determine if it is increased or altered compared to the first or second mutein protease.

Thus, Kolterman *et al.* fails to teach or suggest the elements of any and all of the pending claims.

Waugh *et al.* (Nature Structure Biology 7: 762-765 (2000)

Waugh *et al.* fails to cure these deficiencies. Waugh *et al.* is directed to a description of the structure of granzyme B, and elucidation of the molecular determinants of specificity therefrom. Waugh *et al.* teaches the residues in granzyme B that play a role in determining substrate specificity as deduced from a three dimensional structure of granzyme B in complex with a macromolecular inhibitor, ecotin.

Waugh *et al.* does not teach or suggest any of the instantly claimed methods. Waugh *et al.* fails to teach or suggest a method for producing and indentifying a mutein protease that has increased cleavage activity and/or substrate specificity for any target, including any recited in the claims, nor for increasing cleavage activity and/or substrate specificity of granzyme B for any target. Waugh *et al.* does not teach or suggest any method for modifying granzyme B so that has increased cleavage activity and/or substrate specificity for any target compared to unmodified granzyme B. Hence, there is no teaching or suggestion of

a method for identifying mutant proteases that have increased cleavage activity and/or altered substrate specificity for a target substrate involved in a pathology, which protease could be used to treat the pathology.

Waugh *et al.* teaches the molecular determinates of substrate specificity of granzyme B as determined by analysis of its structure. There is no teaching or suggestion in Waugh *et al.* that modified granzyme B would exhibit altered substrate specificity or increased cleavage activity, nor any teaching or suggestion of methods of modifying granzyme B to do so, nor any modified granzyme B polypeptides. There is no teaching or suggestion of a method of generating mutein granzyme B proteases to have increased cleavage activity and/or altered substrate specificity towards a target substrate compared to a wild-type granzyme B scaffold protease, in particular to a any target substrate involved in a pathology.

Hence, Waugh *et al.* fails to cure the deficiencies in the teachings of Kolterman *et al.* Waugh *et al.*, in fact, is not relevant to the instant claims.

Analysis

The combination of teachings of the cited references does not result in the instantly claimed methods.

The teachings of the references singly or in combination fails to teach or suggest all elements as claimed. Each of independent claims 1, 53, 59 and 63 is directed to a method of producing and identifying a protease mutein that has increased cleavage activity and/or altered substrate specificity for a substrate sequence in a **target protein involved in a pathology** compared to the unmodified protease. As discussed above, neither Kolterman *et al.* nor Waugh *et al.*, singly or together, teaches a method including a step of producing a library of protease muteins of a protease scaffold, where each member of the library has N mutations relative to the wild-type protease scaffold. The library of Kolterman *et al.* contains protease variants specific for a first substrate, and Waugh *et al.* does not teach or suggest a method that employs any library. Furthermore, the library is contacted with a second substrate that contains a specific amino acid sequence resembling the target peptide substrate. In the instantly claimed methods, a mutein protease is identified that has increased cleavage activity and/or substrate specificity for the target compared to the unmodified protease scaffold. In contrast, in the method of Kolterman *et al.*, its library is screened for a mutein that has specificity preferably for the second substrate. Waugh *et al.* does not describe any methods in which any muteins in a library are identified. Thus, the combination of teachings of the cited references fails to teach or suggest identifying muteins that have increased cleavage activity and/or substrate specificity for the target compared to the

unmodified protease scaffold from a library based on scaffold, where each member of the library has N mutations relative to the wild-type protease scaffold. The source of mutein protease is different in the instantly claimed methods form the method of Kolterman *et al.* (or Kolterman *et al.* and Waugh *et al.*,) and resulting mutein protease is identified based on different properties. Thus, the combination of teachings of Kolterman *et al.* and Waugh *et al.* ***cannot*** result in the instantly claimed methods.

Furthermore, each of independent claims 1, 59 and 63 is directed a method of identifying a protease mutein that has **increased cleavage activity and/or altered substrate specificity** for a specified target protein involved in a pathology. **The substrate sequence used in the method is a substrate sequence contained in a *specified* target protein**. For example, each of claims 1 and 59 specify that the target protein is a protein that is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis. Claim 63 recites specific target proteins, for example, caspase 3. There is no teaching or suggestion in any of the cited references of a target protein that is a protein that is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis, nor any specific proteins thereof.

Independent claim 53 also recites that the target protein is one involved in a pathology, and recites additional steps of the method to generate a third mutein protease that contains mutations contained in a first and second identified protease. Thus, claim 53, in addition to containing the steps of the method as discussed above for independent claims 1, 59 and 63 also recites additional steps. The additional steps involve identifying the mutations in protease muteins identified by the method to generate a third protease mutein, and measuring the cleavage activity and/or substrate specificity of the third mutein. None of the cited references, singly or in any combination, teaches a method involving steps of combining identified mutations to generate further muteins.

Thus, the combination of teachings of the Kolterman *et al.* and Waugh *et al.* cannot and does not result in any of the instantly claimed methods. Since a *prima facie* case of obviousness requires that the references, singly or in any combination, must teach all elements as claimed, the Examiner has failed to set forth a case of *prima facie* obviousness.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,

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